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Can Mendelian randomization shift into reverse gear?

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Mendelian randomization (MR), a genetic epidemiological approach, has made substantial inroads into our understanding of the causes, and consequences, of disease *(1, 2)*. Conventionally, MR takes genetic variants associated with an exposure to estimate the causal effect on risk of disease. Availability of large-scale data and hypothesis-free genome-wide analyses has led to discovery of trait-associated genetic variants that surpass stringent thresholds for multiple testing correction, making genome-wide association study (GWAS) discoveries among the most reliable (i.e. among the least prone to false positives) in the scientific literature.

A naïve criticism of GWAS is that the discoveries made therein have limited translational utility. Combining GWAS-identified single nucleotide polymorphisms (SNPs) into genetic instruments under the MR approach, however, offers translational opportunities, yielding notable discoveries for public health and, in the longer-term, pharmaceutical development. More immediate translational opportunities include pharmacogenetics *(3)*, and the emerging use of GWAS for disease prediction *(4)*. In the case of MR, recent examples include identifying that genetic liability to diabetes causes erectile dysfunction *(5)*, and that alcohol consumption may not protect from vascular disease *(6)*. The elucidation of these would be seemingly unachievable under other study designs; e.g., the National Institutes of Health -funded Moderate Alcohol and Cardiovascular Health trial (MACH15; NCT03169530) was abandoned due to perceived influence and bias from Big Alcohol *(7)*. As with all scientific investigations, MR studies can be influenced by unique forms of bias, confounding, and analytical inaccuracies – what differentiates MR from conventional epidemiology is the availability of multiple sensitivity analyses *(8)*, enabling scientists to test many of the implicit assumptions.

In this issue of *Clinical Chemistry*, Mohammadi-Shemirani and colleagues *(9)* have turned MR on its head. They propose taking a summary score of genetic variants robustly associated with estimated glomerular filtration rate (eGFR), a trait used to assess renal function, and have related this to a range of potential biomarkers. Taking 50 eGFR-associated SNPs in combination, they found an association with concentrations of the protein trefoil factor (TFF3). Importantly, cis-acting protein quantitative trait loci (pQTLs) did not demonstrate a causal effect of TFF3 on eGFR, suggesting that the biomarker identified was not a cause, but possibly a consequence of eGFR.

A disease-associated genetic risk score (GRS) would be expected to associate with causes of the disease, consequences of the process leading to the disease, or the disease itself. For example, a GRS for lung cancer that included *CHRNA5* variants related to heaviness of smoking would be expected, therefore, to associate with smoking behavior. It is also possible that a GRS for a disease might identify biomarkers that are influenced by the process leading to disease or the disease itself. In this case, if MR of the biomarker suggests that the biomarker does not cause disease (as in the study by Mohammadi-Shemirani and colleagues*(9)*), the biomarker may be a good candidate to investigate for disease prediction.

What do biomarker-associations of SNPs combined into a GRS that were originally identified for disease (or a precursor of disease) actually mean? We consider these to represent at least seven scenarios (see **Figure 1**), which we elucidate below and in the Figure: note that we do not consider these to be exhaustive. Scenarios 1-4 represent 'real' GRS-to-trait associations, whereas scenarios 5-7 represent potential artifacts either due to analytical method, study design or diagnostic approach to disease.

First, the biomarker association of the GRS may represent a biomarker that causes disease. An example of this would be *PCSK9* genetic variants that associate with coronary heart disease (CHD) at GWAS significance, and which pinpoint LDL-cholesterol as being on the causal pathway to risk of CHD. A GRS-associated biomarker in this scenario may have predictive utility and could also represent a therapeutic target for disease prevention.

Second, disease-GRS associations with a biomarker may represent a consequence of disease, akin to the conventional epidemiological phenomenon of 'reverse causality'. For example, a GRS for CHD is associated with a higher likelihood of receiving statin therapy (analyzed in MR-Base*(10)* on 8th December 2018). A naïve interpretation would be that statin therapy leads to a higher risk of CHD, but of course this is not the case: all individuals with CHD are prescribed statin therapy as first-line treatment.

Third, the biomarker association may arise as a result of *vertical* pleiotropy of a genetic variant or variants that associate with a biomarker (e.g. biomarker X in **Figure 1**) that plays a causal role in disease, but where crucially the GRS-associated biomarker is not causally related to disease (biomarker X_2 in **Figure 1**). For example, genetic variation in *IL6R*, which encodes the interleukin-6 (IL6) receptor (IL6R) is a cause of CHD. Altered activity of the IL6R pathway leads to perturbations in circulating concentrations of both IL6 (which binds to IL6R) and C-reactive protein (CRP) (which IL6, acting through IL6R, modifies). A CHD GRS may identify CRP as associated in this situation as a result of such pleiotropy of the IL6R pathway – note that CRP is not a cause of CHD in this setting. In a similar way, biomarker associations due to vertical pleiotropy of SNPs in the GRS representing pathways arising from disease would lead to a similar association (e.g., the association of a GRS with biomarker Z_2 as a result of vertical pleiotropy through Z in **Figure 1**) .

Fourth, a biomarker association from a GRS may represent *horizontal* pleiotropy *(11)* of one or more genetic variants that also associate with disease. In this situation, one or more genetic variants would associate with the identified biomarker not either through a pathway leading to disease or arising from disease, but from another relationship altogether that is unrelated to disease. A biomarker identified in this scenario is unlikely to have any role in prediction, nor does it represent a valid therapeutic target for disease prevention or treatment.

Fifth, the association may arise due to conditioning for a trait in the original GWAS *(12)*. In using a GRS for type 2 diabetes (T2D), an inverse association with body mass index (BMI) may be identified, which is opposite to the well-recognized causal role of adiposity in T2D. However, conditioning on the original T2D GWAS for BMI would lead to selection of variants that on average increase liability to T2D but are associated with lower BMI.*(13)* On regressing these

SNPs onto phenotypes, an inverse association with BMI may be identified, purely as a result of the analytical model of the original T2D GWAS.
Sixth, the association may be induced by selection biases affecting cases or controls. For example, if cases are identified from a screening program and controls are from the general population, selection biases into a screening program might identify associations of the GRS with socioeconomic factors such as years of education.
Seventh, the biomarker leads to disease diagnosis but the biomarker itself is not causal. For example, increased prostate specific antigen (PSA) concentration is used to diagnose prostate cancer. Thus a GRS of prostate cancer may associate with PSA even in the absence of a causal effect of PSA on prostate cancer.

In the current article *(9)*, the focus was on the application of a pre-disease GRS to identify novel biomarkers for prediction. As the authors note, the ideal biomarker for prediction is one that is independently and strongly associated with a disease and which makes meaningful improvements to disease prediction – i.e. it need not be causal. In this case, out of the seven scenarios above, when might a biomarker be useful for prediction? Biomarker associations arising from horizontal pleiotropy of a genetic instrument is unlikely to have clinical utility, nor is an association that is induced by adjusting for a trait in the discovery GWAS, when there are selection biases present, or when diagnosis is based on a non-causal biomarker. The authors *(9)* tease apart potential sources of bias, and ultimately, are able to show that inclusion of TFF3 leads to modest improvements in disease prediction: the *c*-statistic increasing from 0.59 to 0.60. Such measures of discrimination may be insensitive to small, but meaningful, increments in predictive utility *(14)*. Comparatively, recent studies that incorporated genetic variants for prediction of CHD led to similar changes in area-under -the-curve, from *c*-statistics of 0.67 for conventional risk factors to 0.70 with inclusion of 1.7 million genetic variants *(4)*.

When might combinations of measured phenotype and/or its genotypic variation be useful in prediction? When a biomarker itself is causally related to disease, phenotypic measurements are likely to be useful beyond genotype, as the measured trait will additionally capture environmental variation (as it is the combined genetic and environmental variation in the causal biomarker that ultimately leads to disease). In this same scenario, genotype will also be useful in addition to the measured phenotype as genotype represents a measure of lifelong exposure. When the biomarker is a consequence of disease (i.e. the association arises from reverse causality), measuring the trait is of importance as it gives a dynamic 'read out' of disease status. Finally, when a biomarker is non-causal in disease, including genotype in the model may increase the predictive utility of the biomarker, through the principle of so-called "biomarker de-Mendelization", by maximizing the non-genetic variation in the phenotype *(15)*.

With burgeoning technological advances in high throughput phenotyping of omics down-stream of the invariant genome (notwithstanding CRISPR), unparalleled opportunities exist for repurposing and adapting the principles of MR to new approaches that may lead to novel prospects for the translation of GWAS discoveries. Such applications are expected to lead to

advances not only in elucidating the causes and consequences of disease, but as in the current study, to potential improvements in disease prediction.

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Disclosures

None

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Figure Legends

Figure 1. Potential scenarios of biomarker associations of a genetic risk score for disease. The seven scenarios are described in the main text. Purple cloud call-outs elucidate whether the biomarker is likely to have a role in disease prediction and/or whether it represents a potential therapeutic target for the treatment and/or prevention of disease. GRS = genetic risk score.